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Quantum Dot Labeling of Stem Cells during Proliferation and Differentiation

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1 Introduction

Labeling cells and tracking their fate in vitro, in vivo and in real time is an imperative tool for tissue engineers upon fabrication of therapeutic implants in order to determine the partial contribution of multiple cell types in complex tissues as well as their migration and maturation. Stem cells rapidly proliferate and potentially differentiate making labeling and tracking of these cells complex yet indispensable. There are currently 3 types of cell labeling probes: organic dyes, fluorescent proteins and quantum dots. Organic dyes readily photobleach and lose fluorescence, and therefore are only useful for short-term cell labeling. Green Fluorescent Proteins (GFPs) suffer from a number of intrinsic deficiencies such as sensitivities to proteolytic enzymes, difficulty in labeling multiple cell lineages and may overlap with autofluorescence signal during in vivo cell tracking. Quantum dots (QDs) are small light-emitting semiconductor particles, typically in the size range of 2-10 nm. QDs are photostable and maintain fluorescence intensity in cell culture for prolonged time. The narrow emission spectrum and broad excitation spectrum of QDs enable the viewing of multiple colors by single wavelength activation. Previous studies have shown the capacity of labeling and tracking rapidly dividing tumor cells in vivo and in real time using quantum dots. Given the high proliferative nature of human mesenchymal stem cells (hMSCs) and their phenotypic changes during differentiation, the present study was designed to determine the efficacy of labeling on hMSCs during proliferation and differentiation with bioconjugated quantum dots.

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2 Materials and Methods

Isolation and Culture of Human Mesenchymal Stem Cells (hMSCs). Human mesenchymal stem cells (hMSCs) were isolated from bone marrow samples by negative selections and cultured with DMEM supplemented with 10% fetal bovine serum (FBS), and 1% penicillin and streptomycin with medium changes every 3-4 days.

Preparation of Bioconjugated Quantum Dots (QDs). Zinc sulphide (ZnS) capped cadmium selenide (CdSe) quantum dots with functionalized carboxyl surface groups (em: <450 nm, ex: 600 ± 10 nm - Fort Orange dots, Evident Tech, Troy, NY) were conjugated to CGGGRGD through covalent bonding by using EDC. The RGD peptides were allowed to react with QDs bound to Sulfo-NHS for 2 hrs at RT and then stored at 4ºC overnight.

hMSC labeling with Quantum Dots. hMSCs (45,000 cells/well) were incubated with the bioconjugated QDs (0.5, 5, 20, 30, or 50 nM) for 5 min, 30 min, 2 hrs, or 16 hrs. Following repetitive washing of QD-labeled hMSCs and removal of unbound QDs, the QD-labeled cells were culture-expanded in DMEM for up to 22 days.

hMSC lineage derivatives labeling with Quantum Dots. After labeling with QD bioconjugates, a subpopulation of hMSCs were induced to differentiate into osteogenic, chondrogenic and adipogenic cells. For osteogenic differentiation, supplements containing 100 nM dexamethasone, 0.05 mg/mL ascorbic acid, and 0.01 M β-glycerophosphate were used. Chondrogenic differentiation occured in pellet culture in serum-free, high glucose DMEM supplemented with 1% ITS+ premix, 100 nM dexamethasone, 50 μg/mL ascorbic acid, 50 μg/mL ascorbate, 40μg/mL L-

proline, 10 ng/mL TGF-β3, 1% Penn/strep. Adipogenic differentiation of hMSCs was induced by exposure 50 nM Dexamethasone, 10 mM insulin, and 5 mM isobutyl-methylxanthine.

3 Results

hMSC labeling by Quantum dots. QDs were incubated with hMSCs at 0.5, 5, 20 and 50 nM concentrations. A 20 nM QD concentration showed slightly inferior labeling efficacy to 50 nM. Accordingly, 30 nM concentration was utilized in additional experiments (data not shown). QDs were incubated with hMSCs at the selected 30 nM concentration for 5 min, 30 min, 2 hrs and 16 hrs. A 16 hr (overnight) incubation yielded the effective labeling and was utilized in additional experiments (data not shown). QDs remained visible at 4, 7 and 22 days of hMSC culture expansion (**Fig. 1**). Cell proliferation and viability were apparent and confirmed by quantitative DNA content (**Fig. 2**) showing lack of statistically significant differences between QD-labeled hMSCs and unlabeled hMSCs of the same subpopulation. In comparison with unlabeled hMSCs (6.5%), fluorescent cell counting demonstrated high yield of QD fluorescent-hMSCs (96%) post-labeling (data not shown). QDs did not affect osteogenic differentiation, as there was no significant differences in ALP contents and calcium production between QD-labeled and unlabeled hMSCs (**Fig. 3**). Chondrogenic differentiation was demonstrated by positive alcian blue staining of QD-labeled or unlabeled hMSCs (**Fig. 4**). No statistically significant differences in glycosaminogalycan (GAG) contents between QD-labeled hMSCs and unlabeled hMSCs was observed (**Fig. 4F**). Formation of intracellular lipid vacuoles in QD-labeled hMSCs during adipogenic differentiation was observed indicating no adverse effects of QD-labeling (**Fig. 5**). Oil-red O staining showed similar levels of adipogenesis formation with or without QD labeling (**Fig. 5D, E**). Additionally, no statistically significant differences were found in glycerol contents between QD-labeled and unlabeled hMSCs (**Fig. 5F**).

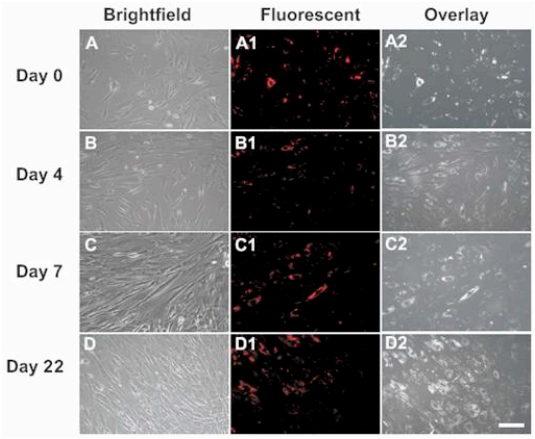

Figure 1 : Human mesenchymal stem cells (hMSCs) labeled with bioconjugated quantum dots (QDs). QDs remained visible at 4, 7 and 22 days of hMSC culture expansion. Scale bar: 30 μm.

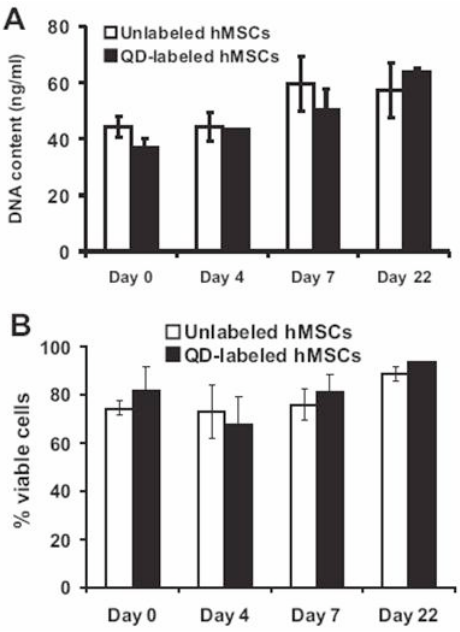

Figure 2 : DNA and cell viability of human mesenchymal stem cells (hMSCs) labeled with bioconjugated quantum dots (QD) **A:** DNA contents lacked statistically significant differences between QD-labeled hMSCs and unlabeled hMSCs. **B:** Cell viability lacked statistically significant differences between QD-labeled hMSCs and unlabeled hMSCs.

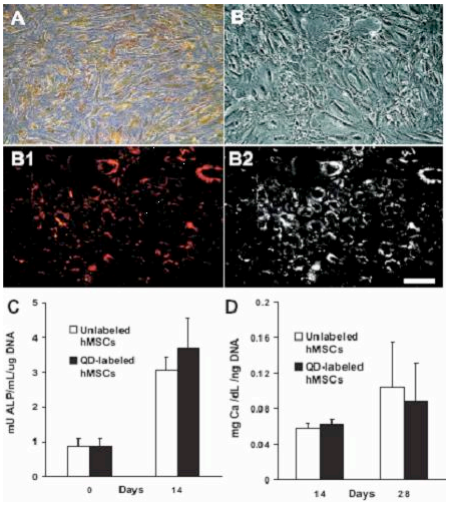

Figure 3 : Osteogenic differentiation of human mesenchymal stem cells (hMSCs) labeled with bioconjugated quantum dots (QDs). **A:** Expression of alkaline phosphatase (ALP) during osteogenic differentiation of QD-labeled hMSCs. **B-B2:** QDs remained in hMSCs during osteogenic differentiation. **C** and **D:** No significant differences in ALP contents and calcium production between QD-labeled and unlabeled hMSCs, respectively. Scale: 30 µm.

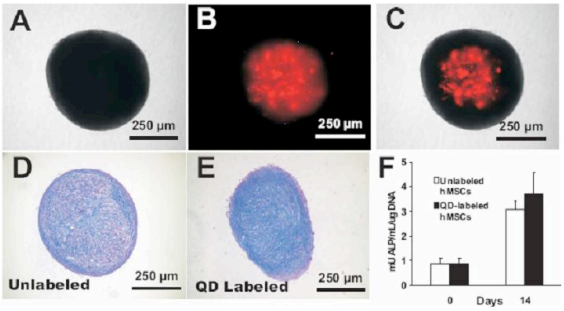

Figure 4 : Chondrogenic differentiation of human mesenchymal stem cells (hMSCs) labeled with bioconjugated quantum dots (QDs). **A-C:** QD labeling of hMSCs during chondrogenic differentiation in pellet culture. **D** and **E:** Positive alcian blue staining of QD-labeled or unlabeled hMSCs during chondrogenic differentiation. **F:** No statistically significant differences in glycosaminogalycan (GAG) contents between QD-labeled hMSCs and unlabeled hMSCs.

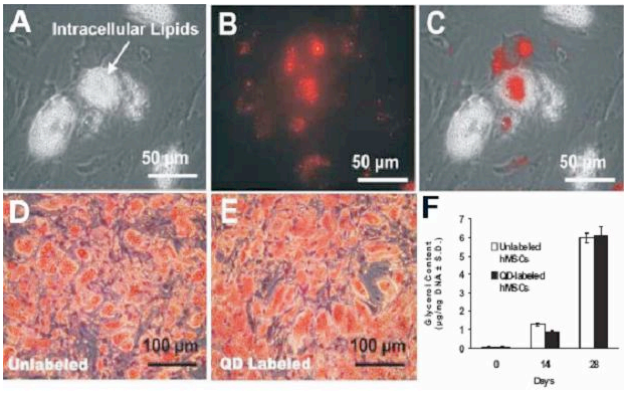

Figure 5 : Adipogenic differentiation of human mesenchymal stem cells (hMSCs) labeled with bioconjugated quantum dots (QDs). **A-C:** Formation of intracellular lipid vacuoles in QD-labeled hMSCs during adipogenic differentiation. **D** and **E:** Oil-red O staining showing adipogenesis formation without (**D**) or with (**E**) QD labeling. **F:** No statistically significant differences in glycerol contents between QD-labeled and unlabeled hMSCs.

4 Conclusion

The present findings demonstrate that bioconjugated quantum dots (QDs) are capable of labeling human mesenchymal stem cells (hMSCs) during population doubling for the tested 22 days. Moreover, during the differentiation of hMSCs into chondrogenic, osteogenic and adipogenic cells, QDs continued to reside in offspring cells without substantially affecting their differentiation behavior. These findings suggest that bioconjugated quantum dots may be an effective probe for labeling stem cells during differentiation into multiple lineages.

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